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Presence and Significance of Human Parvovirus B19 DNA in Synovial Membranes and Bone Marrow From Patients With Arthritis of Unknown Origin

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Acute rheumatologic symptoms are frequently associated with human parvovirus B19 (B19) infections. A nested PCR (nPCR) assay was used to test for the presence of parvovirus B19 DNA in synovial fluid and/or synovial membrane specimens obtained from a total of 90 patients with arthritis of unknown origin. Whereas only one out of 73 synovial fluid samples were found positive, 15 (16.7%) out of 90 patients had parvovirus B19 DNA in the synovium. B19 virus DNA was detected in nine bone marrow aspirates subsequently obtained from these 15 patients (60%). Whereas each one of the 15 corresponding blood samples contained anti-B19 IgG antibody, none contained anti-B19 IgM antibody and only one was positive for B19 virus DNA. The blood and synovial fluid samples that contained B19 virus DNA were obtained from the same patient, who also had B19 DNA in synovium and bone marrow. For one patient, two distinct synovial membrane specimens collected 10 months apart tested positive for B19 virus DNA. Parvovirus B19 DNA was also detected in synovial tissue of one out of nine nonarthritic patients serving as control group, who also had anti-B19 IgG circulating antibody. These data illustrate that human parvovirus B19 may persist in bone marrow and synovial tissues of patients with arthritis of unknown origin. In contrast, persistence of B19 virus DNA in synovial fluid is rare. The significance of parvovirus B19 DNA in synovium of healthy patients has to be established. ***J. Med. Virol. 56:199–204, 1998.***

KEY WORDS: PCR; polymerase chain reaction; persistence; diagnosis; synovial fluid

INTRODUCTION

Human parvovirus B19 is the etiological agent of erythema infectiosum (EI), a common febrile illness often characterized by a maculopapular rash [Anderson et al., 1983]. Among the possible complications of parvovirus B19 infection, which are mainly described in adults [for review see Cassinotti, 1995], arthralgia and arthritis are the most common [Reid et al., 1985; White et al., 1985; Cassinotti et al., 1995]. The arthritis is usually symmetrical and polyarticular involving primarily the small joints of the hands, wrists, ankles, and knees. Acute arthritis as the most severe manifestation usually resolves within a few weeks. However, it may take a prolonged chronic course in about 20% of cases [Woolf et al., 1989], a pattern possibly associated with persistence of the virus [Foto et al., 1993; Jawad, 1993; Nocton et al., 1993; Söderlund et al., 1997]. If only patients with clinical signs of EI are considered, joint pain has been reported in 5% of children up to nine years of age, but occur in 77% of patients older than 20 years [Ager et al., 1966]. Joint pain and swelling occur more frequently in adult women than men. However, individuals infected with human parvovirus B19 may present without the common signs of EI such as rash, 26% of infected adults being totally asymptomatic [Woolf et al., 1989]. Thus, the exact incidence of parvovirus B19 infection in patients with arthralgia or arthritis remains unknown and is probably underestimated. A higher incidence of parvovirus B19 infection in children with joint complaints was recently discussed [Nocton et al., 1993].

In order to study the role of human parvovirus B19 in patients suffering from arthritis of unknown origin, the presence of human parvovirus B19 DNA was tested by nested PCR in synovial fluid and synovial membranes

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collected by needle arthroscopy. In addition, blood and bone marrow samples obtained from patients with B19 DNA detected in synovial membranes were also examined.

MATERIALS AND METHODS

Specimens

Patients with unexplained arthritis. A total of 90 patients with idiopathic arthritis, 44 males and 46 females, between 15 and 70 years of age (mean: 38.4 years) were investigated. Synovial fluid and synovial membrane specimens collected by needle arthroscopy were available for 73 and 90 patients, respectively. Bone marrow and blood samples were subsequently obtained following informed consent from 15 patients whose synovial membranes were shown to contain human parvovirus B19 DNA.

Control patients. As controls, synovial fluid and synovial membrane specimens were obtained from nine patients without arthritis who underwent knee surgery. Four of these control patients had anti-B19 IgG antibody in their blood as evidence of past parvovirus B19 infection and five were negative for anti-B19 antibody.

DNA Extraction

Parvovirus B19 DNA was obtained from serum by heating an aliquot at 94°C for 10 min and from synovial fluid and bone marrow specimens by treatment with proteinase K (lysis buffer: 10 mM tris-HCl, pH8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 1% Laureth 12 or 0.5% Tween 20; 100 μg/ml fresh proteinase K) as previously described [Cassinotti et al., 1993]. DNA was purified from synovial membrane specimens using a commercial spin column procedure according to the supplier's protocol (QIAamp tissue kit, Qiagen, Basel, Switzerland). Briefly, the tissue samples were digested at 55°C overnight in a proteinase K stock solution. Following digestion, the samples were adjusted to binding conditions and loaded onto a spin column. DNA was subsequently adsorbed onto the silica membrane of the column, washed and eluted in a total of 400 μl elution buffer preheated to 70°C.

PCR Amplification

Parvovirus B19 nested PCR. Following DNA extraction from synovial fluid, synovial membrane, blood or bone marrow specimens, a fragment of the DNA sequence coding for the major capsid protein (VP2) of parvovirus B19 was amplified by nested PCR (nPCR) as previously described [Cassinotti et al., 1997]. The first amplification consisted of 35 cycles carried out at an annealing temperature of 45°C using the outer primers TJI (direct primer, nucleotides 3775–3792, 5′-TTCTTTTCAGCTTTTAGG-3′) and R4154 (reverse primer, nucleotides 4154–4171, 3′-TTTATACAGTGTCCTTAT-5′) numbered according to Shade et al. [1986]. The inner nested primers 968 (direct primer, nucleotides 3818–3837, 5′-TATAAGTTTCCTCCAGTGCC-3′) and TJII (reverse primer, nucleotides 3956–3975, 3′-GTACTTCTGGTACGTTAAGT-5′) were used for the second amplification, consisting of 35 cycles undertaken at an annealing temperature of 60°C. The 158-base-pair (bp) diagnostic fragment amplified with the inner primers 968 and TJII was subsequently detected by agarose gel electrophoresis. Each sample was tested in duplicate. A 10^8-fold dilution of a viremic reference serum (N3787) containing approximately 10 to 100 parvovirus B19 genome copies was used as positive control. In order to test for the presence of inhibitors of the PCR reaction, distinct aliquots of the synovial fluid, bone marrow, and blood specimens were spiked with the diluted reference serum prior to DNA extraction. Negative controls consisting of phosphate buffered saline (PBS) were extracted concomitantly with the diagnostic specimens to monitor the lack of contamination during the extraction step. Additional negative controls were included in each PCR run to test for the absence of carryover contamination.

TABLE I. Detection of Parvovirus B19 in Synovial Membranes and Synovial Fluid Specimens From Patients With Idiopathic Arthritis

Synovial membrane specimens		Synovial fluid specimens	
B19-DNA	Number of patients (%)	B19-DNA	Number of patients (%)
Positive	15 (16.7%)	Positive	1 (1.4%)
Negative	75 (83.3%)	Negative	72 (98.6%)
Total	90 (100.0%)	Total	73 (100.0%)

β-Globin PCR. In order to test the absence of inhibitors and to assess the suitability of DNA extracted from synovial membranes as substrate for PCR amplification, a 268 bp fragment of the β-globin gene was amplified using the primers PC04 (direct primer, 5′-CAACTTCATCCACGTTCACC-3′) and GH20 (reverse primer, 3′-CATGGACAGGAACCGAGAAG-5′) as described by Bauer et al. [1991]. Briefly, 10 μl of extracted DNA were added to 90 μl of amplimix prior to amplification (35 cycles; denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 60 sec). The PCR products were subsequently resolved by agarose gel analysis. DNA extracted from human liver was used as positive control. Negative controls consisted of PBS samples extracted in parallel.

Serological Tests

Commercial enzyme immunoassays or immunofluorescence assays based on the use of parvovirus B19 recombinant capsid proteins were used to test for the presence of anti-B19 IgM and IgG antibodies in serum (Parvovirus B19 IgM and IgG enzyme immunoassays, Parvovirus B19 immunofluorescent assay, Biotrin, Dublin, Ireland).

TABLE II. Summary of Data From Patients With B19 Genome in Synovial Membranes

Patient number (initials)	Sex	Age (years)	Joint examined	Synovial membrane B19 DNA	Synovial fluid B19 DNA	Bone marrow B19 DNA	Blood B19 DNA	Blood IgM	Blood IgG
1 (BL)	F	44	Knee	Positive	Negative	Negative	Negative	Negative	Positive
2 (BS)	F	37	Knee	Positive	Negative	Negative	Negative	Negative	Positive
3 (BK)	F	33	Knee	Positive	Negative	Positive	Negative	Negative	Positive
4 (DS)	M	31	Knee	Positive	Negative	Negative	Negative	Negative	Positive
5 (FE)	F	48	Knee	Positive	Negative	Positive	Negative	Negative	Positive
6 (GC)	M	28	Ankle	Positive[a]	Negative	Negative	Negative	Negative	Positive
7 (HM)	M	38	Ankle	Positive	Negative	Positive	Negative	Negative	Positive
8 (JC)	F	37	Knee	Positive	Negative	Negative	Negative	Negative	Positive
9 (MR)	M	24	Knee	Positive	Negative	Negative[b]	Negative	Negative	Positive
10 (PC)	F	53	Knee	Positive	Negative	Positive	Negative	Negative	Positive
11 (SM)	M	33	Knee	Positive	Negative	Positive	Negative	Negative	Positive
12 (SS)	F	23	Knee	Positive	Negative	Positive	Negative	Negative	Positive
13 (SB)	F	28	Wrist	Positive	Negative	Positive	Negative	Negative	Positive
14 (VTG)	M	44	Knee	Positive	Negative	Positive	Negative	Negative	Positive
15 (VTJ)	F	41	Knee	Positive	Positive	Positive	Positive	Negative	Positive

[a]B19 DNA also detected in synovium from knee collected 10 months later.
[b]PCR inhibited in this sample.

RESULTS

Detection of Parvovirus B19 DNA in Synovial Membrane and Synovial Fluid Specimens

The presence of parvovirus B19 genome was investigated in synovial fluid and/or synovial membrane specimens collected by needle arthroscopy from 90 patients with arthritis of unknown origin (Tables I and II; the clinical manifestations and symptoms will be presented elsewhere). Parvovirus B19 DNA was detected by nPCR in synovial membranes in 15 out of 90 patients (16.7%). Of these patients, nine were women between 23 and 53 years (mean age: 38.2 years) and six were men between 24 and 44 years (mean age: 33.0 years). For one of these patients (Table II, patient GC), parvovirus B19 DNA was detected again in a second synovial membrane specimen collected 10 months after the first one. Among synovial fluid specimens available for 73 patients, only one (1.4%) contained parvovirus B19 DNA. This patient also had B19 DNA in the corresponding synovial membrane sample. In patients with B19 DNA detected in synovial membranes, the joints of the knees, fingers, and toes were the most frequently involved. Although this distribution of joints affected is frequently encountered in the course of a parvovirus B19 infection, it was similar in the B19 DNA-negative patients.

Detection of Parvovirus B19 DNA in Bone Marrow and Blood Specimens

To further document the nature of the parvovirus B19 infection in the 15 arthritic patients with B19 DNA present in synovial membranes, bone marrow and blood samples were collected following informed consent (Tables II and III). Parvovirus B19 DNA was detected by nPCR in bone marrow of 9 (60%) of these 15 patients. Among the remaining six bone marrow specimens that tested negative for B19 virus DNA, one contained inhibitors of the PCR reaction possibly leading to a false negative PCR result. Only 1 (6.7%) of the corresponding 15 blood samples tested positive for B19 DNA. This patient (Table II, VTJ) also had B19 virus DNA detected in bone marrow, synovial fluid, and synovial membrane. Whereas circulating anti-B19 IgG antibody was detected in the blood of each of the above 15 patients (100%), none were positive for anti-B19 IgM antibody. This serological pattern is considered typical of a past parvovirus B19 infection. Among the remaining 75 patients whose synovial membranes did not contain B19 DNA, 42 (56%) had anti-B19 IgG antibodies. The difference in the seroprevalence of anti-B19 IgG antibody between the two groups is statistically significant ($P = 0.001$; Fisher's exact test).

PCR Detection of B19 DNA in a 33-Year-Old Man With Knee Arthritis

Figure 1 shows the results obtained in synovial membrane, synovial fluid, bone marrow, and blood specimens from a 33-year-old man with arthritis of unknown etiology. He developed acute synovitis of the left knee two weeks after flulike upper respiratory symptoms, fever, and diarrhea. No malar or subtle rash was reported. His erythrocyte sedimentation rate was 42 mm/hr and the C-reactive protein concentration was 38 mg/l (norm <10). Blood cell count was normal; rheumatoid factor, antinuclear antibody, antibodies, and stool culture for enteropathic bacteria were all negative. Joint fluid cell count was 6.0×10^3/ml; no crystals or microorganisms were detected. PCR was negative for chlamydia and other bacterial DNA. The presence of parvovirus B19 DNA was confirmed by a 158 bp diagnostic amplicon in a synovial knee membrane biopsy (lane 3) but not in synovial fluid (lane 1). The genome of parvovirus B19 was also present in bone marrow (lane 6) but not in a corresponding blood sample (lane 4), which contained anti-B19 IgG antibody but no anti-B19 IgM antibody. For technical reasons the blood and bone marrow samples were collected six months after needle arthroscopy. Since no renewed needle arthros-

TABLE III. Detection of Parvovirus B19 DNA in Bone Marrow and Blood Specimens From 15 Patients With B19 DNA in Synovial Membranes

Bone marrow B19 DNA	Blood B19 DNA	Number of patients (%)	Serological status[a]
Positive	Negative	8 (53.3%)	8 IgM– and IgG+
Negative	Positive	0 (0.0%)	
Positive	Positive	1 (6.7%)	1 IgM– and IgG+
Negative	Negative	6 (40.0%)[b]	6 IgM– and IgG+

[a]IgM denotes anti-B19 IgM antibody; IgG, anti-B19 IgG antibody.
[b]PCR inhibited in one bone marrow sample.

copy was undertaken, it is not known whether B19 virus DNA was still present in the synovium at that time. The absence of inhibitors of the PCR reaction in synovial fluid, blood, and bone marrow was demonstrated in aliquots spiked with parvovirus B19 (lanes 2, 5, and 7). The suitability of DNA extracted from synovial membrane as substrate for PCR amplification was demonstrated by amplification of a 268 bp fragment of the β-globin gene (lane 8).

Detection of Parvovirus B19 DNA in Synovial Membranes and Synovial Fluid Specimens From Control Patients

Among nine nonarthritic control patients for whom synovial membrane and synovial fluid specimens were available, one had B19 DNA in synovial membrane together with anti-B19 circulating IgG antibody. None of the synovial fluid specimens was positive for B19 DNA. No bone marrow specimens were available from patients in the control group. The β-globin polymerase chain reaction was not inhibited in any of the control synovial membrane specimens examined. Similarly, no inhibitors were detected in the synovial fluid specimens spiked with B19 virus.

DISCUSSION

The aim of this study was to investigate the possible role of human parvovirus B19 in patients suffering from arthritis of unknown origin. Whereas B19 virus DNA was detected in synovial membranes from 15 (16.7%) of the 90 patients investigated, only 1 (1.4%) out of 73 synovial fluid specimens was positive. This result has to be viewed against a background of reports that either documented the presence of parvovirus B19 DNA in synovium, synovial fluid, and synovial fluid cells from patients with various arthropathies [Dijkmans et al., 1988; Kandolf et al., 1989; Kerr et al., 1995a; Nikkari et al., 1995], or failed completely to detect parvovirus B19 DNA in synovial fluid or synovial fluid cells obtained from patients diagnosed with early rheumatoid arthritis [Nikkari et al., 1994]. Our data strongly suggest that synovial membrane specimens are more appropriate than synovial fluid to demonstrate parvovirus B19 DNA in joint material of patients with idiopathic arthritis. Preferred use of synovial tissue instead of synovial fluid to demonstrate microbial DNA has also been documented for other organisms such as *Chlamydia trachomatis* [Branigan et al., 1996].

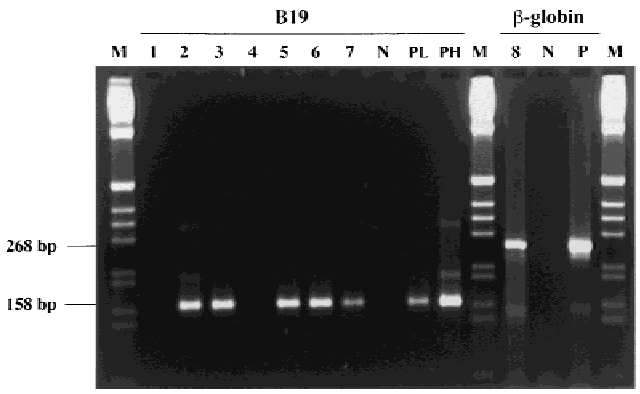

Fig. 1. PCR detection of B19 DNA in a 33-year-old man with idiopathic arthritis (patient SM). Parvovirus B19 DNA was detected in synovial membrane (lane 3) and bone marrow (lane 6) but not in synovial fluid (lane 1) or blood (lane 4). The absence of inhibitors was demonstrated by the obtention of a 158 bp amplicon in spiked aliquots of synovial fluid, blood, and bone marrow (lanes 2, 5, and 7, respectively). The absence of inhibitors in synovial membrane was demonstrated by amplification of a 268 bp fragment of the β-globin gene (lane 8). PL denotes positive control for B19 DNA low (10^{-8}-fold dilution of B19 reference serum); PH, positive control for B19 DNA high (10^{-7}-fold dilution of B19 reference serum); N, negative control β-globin; P, positive control β-globin; M, marker.

In the small control group, one individual with circulating anti-B19 IgG antibody had B19 virus DNA in joint tissue. The presence of B19 virus DNA in synovial tissue from nonarthritic patients has been recently documented in a study searching for markers of human parvovirus B19 infection in children with juvenile chronic arthritis [Söderlund et al., 1997]. In a control group of 27 young adults with joint trauma, 13 (48%) individuals had B19 virus DNA in synovial tissue together with circulating anti-B19 IgG antibody. With a seroprevalence of 48% (13/27), each control individual with anti-B19 IgG antibody also had B19 virus DNA detected in synovial membrane. The high incidence of B19 virus DNA in synovial membranes reported by Söderlund et al. [1997] for the control patients contrasts with our results. Methodological differences such as the size of the synovial membrane specimens, which is extremely small in our case, may provide an explanation. The presence of parvovirus B19 DNA in the synovium of arthritic as well as of control patients thus indicates that B19 virus DNA may persist in synovial membrane of individuals with serological evidence of past parvovirus B19 infection. Persistence of B19 virus DNA in synovium was further substantiated by detection of the viral DNA in two different specimens obtained from the same arthritic patient at a 10-month interval (Table II, patient GC).

During PCR a portion of B19 virus genome is amplified, thus it is not known whether the whole genome of an actively replicating virus is present or if only nucleolytic fragments are detected. In the case of acute parvovirus B19 arthritis the symptoms correlate with the beginning of the immune response and the possible

deposition of immune complexes, which indicates that the rheumatologic manifestations may be an indirect consequence of the infection. Usually parvovirus B19 infections are self-limited and of short duration in the immunocompetent individual. They may, however, take a chronic course possibly associated with persistence of the virus in the organism and recurrence of the joint symptoms [Faden et al., 1992; Foto et al., 1993; Kerr et al., 1995b; Sasaki et al., 1995]. Although synovial membrane cells do not appear to be permissive for active parvovirus B19 replication, abortive infection cannot be excluded, since B19 virus DNA was detected in the cellular fraction following infection of cultured human synovial cells [Miki and Chantler, 1992]. Another cellular compartment that may harbor the virus in persistently infected individuals is bone marrow. Persistence of B19 virus DNA at this location for at least two years following the onset of acute symptoms has been previously documented for four patients who developed chronic joint symptoms [Foto et al., 1993]. In a recent study aimed at investigating the persistence of human parvovirus B19 DNA in the general population [Cassinotti et al., 1997], we have indeed shown that 11% of healthy bone donors with serologically documented past B19 virus infection had B19 DNA in bone marrow. In the present report testing was carried out under the same conditions and parvovirus B19 DNA has been detected in bone marrow from 9 (60%) of the 15 patients with B19 DNA-positive synovial membranes. Prolonged viremia is rare since only 1 of these 15 patients also had B19 virus DNA in the circulating blood. A past and by accepted diagnostic standards resolved parvovirus B19 infection was further documented in these 15 patients by the presence of circulating anti-B19 IgG antibody and the absence of anti-B19 IgM antibody. These results indicated that patients with arthritis of unknown origin appear to carry parvovirus B19 DNA in the bone marrow more often than healthy patients. The higher rate of B19 DNA detection observed in bone marrow from arthritis patients is indeed statistically significant ($P = 0.002$; Fisher's exact test). For these individuals the joint symptoms may be linked to the onset of immune mechanisms triggered by reactivation of a latent parvovirus B19 infection in bone marrow. It could also be speculated that reactivation may be favored by genetic factors of the host. Evidence for reactivation of persistent parvovirus B19 infection has been demonstrated in a normal subject [Sasaki et al., 1995] and in a four-year-old boy with acute lymphocytic leukemia [Coulombel et al., 1989].

In conclusion, the data obtained in the present study indicate that human parvovirus B19 DNA may persist in synovial membranes and bone marrow, but not in synovial fluid, of patients with unclassified arthritis who have serological evidence of past parvovirus B19 infection. Whereas parvovirus may be either directly or indirectly responsible for the rheumatologic manifestations observed in at least some of the patients, a causal relationship needs to be established.

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